

**What is claimed is:**

1. An adzyme for inhibiting receptor-mediated signaling activity of an extracellular substrate polypeptide, the adzyme being a fusion protein comprising a protease domain that catalyzes the proteolytic cleavage of at least one peptide bond of the substrate polypeptide so as to inhibit the receptor-mediated signaling activity of the polypeptide, and a targeting domain that reversibly binds with an address site on said substrate polypeptide, wherein said targeting domain and said protease domain are discrete and heterologous with respect to each other.
2. The adzyme of claim 1, wherein said adzyme is resistant to cleavage by said protease domain.
3. The adzyme of claim 1, wherein said protease domain is a zymogen.
4. The adzyme of claim 1, wherein said protease domain is selected from among: a serine proteinase and a metalloproteinase.
5. The adzyme of claim 1, wherein said adzyme is purified from a cell culture in the presence of a reversible protease inhibitor that inhibits the protease activity of the protease domain.
6. The adzyme of claim 1, wherein said adzyme has one or more properties, with respect to the reaction with said substrate, of (a) a potency at least 2 times greater than the protease domain or the targeting moiety alone; (b) a  $k_{on}$  of  $10^3 \text{ M}^{-1}\text{s}^{-1}$  or greater; (c) a  $k_{cat}$  of  $0.1 \text{ sec}^{-1}$  or greater; (d) a  $K_D$  that is at least 5 fold less than the  $K_m$  of the protease domain; (e) a  $k_{off}$  of  $10^{-4} \text{ sec}^{-1}$  or greater, (f) a catalytic efficiency at least 5 fold greater than the catalytic efficiency of the protease domain alone, (g) a  $K_m$  at least 5 fold less than the  $K_m$  of the protease domain alone, and/or (h) an effective substrate concentration that is at least 5 fold greater than the actual substrate concentration.
7. The adzyme of claim 6, wherein the potency of the adzyme is at least 5 times greater than the protease domain or the targeting moiety alone.
8. The adzyme of claim 6, wherein the  $k_{on}$  is  $10^6 \text{ M}^{-1}\text{s}^{-1}$  or greater.
9. The adzyme of claim 6, wherein the  $k_{cat}$  is  $10 \text{ sec}^{-1}$  or greater.

10. The adzyme of claim 6, wherein the  $K_D$  is at least 50 fold lower than the  $K_m$  of the protease domain.
11. The adzyme of claim 6, wherein the  $k_{off}$  is  $10^{-3} s^{-1}$  or greater.
- 5 12. The adzyme of claim 6, wherein the catalytic efficiency is at least 20 fold greater than that of the protease domain alone.
- 10 13. The adzyme of claim 6, wherein the  $K_m$  is at least 20 fold less than that of the protease domain alone.
14. The adzyme of claim 1, wherein said linker is an unstructured peptide.
- 15 15. The adzyme of claim 1, wherein said linker includes one or more repeats of Ser<sub>4</sub>Gly or SerGly<sub>4</sub>.
16. The adzyme of claim 1, wherein said linker is selected to provide steric geometry between said protease domain and said targeting domain such that said adzyme is more potent than said protease domain or targeting moiety with respect to the reaction with said substrate.
- 20 17. The adzyme of claim 1, wherein said linker is selected to provide steric geometry between said protease domain and said targeting moiety such that said address moiety presents the substrate to the enzymatic domain at an effective concentration at least 5 fold greater than would be present in the absence of the address moiety.
- 25 18. The adzyme of claim 1, wherein the fusion protein is a cotranslational fusion protein encoded by a recombinant nucleic acid.
- 30 19. The adzyme of claim 1, wherein the adzyme is resistant to autocatalyzed proteolysis.
20. The adzyme of claim 19, wherein the adzyme is resistant to autocatalyzed proteolysis at an adzyme concentration that is about equal to the concentration of adzyme in a solution to be administered to a subject.
- 35 21. The adzyme of claim 6, wherein said substrate is present in biological fluid of an animal.

22. The adzyme of claim 21, wherein said biological fluid is blood or lymph.
23. The adzyme of claim 21, wherein said substrate is a polypeptide hormone, a  
5 growth factor and/or a cytokine.
24. The adzyme of claim 21, wherein said substrate is selected from among: four-helix  
bundle factors, EGF-like factors, insulin-like factors,  $\beta$ -trefoil factors and cysteine  
knot factors.
- 10 25. The adzyme of claim 21, wherein said substrate is an inflammatory cytokine and  
said enzyme construct reduces the pro-inflammatory activity of said polypeptide  
factor.
- 15 26. The adzyme of claim 1, wherein the targeting domain is an antibody or  
polypeptide(s) including an antigen binding site thereof.
27. The adzyme of claim 1, wherein the targeting moiety is selected from the group  
consisting of a monoclonal antibody, an Fab and F(ab)<sub>2</sub>, an scFv, a heavy chain  
20 variable region and a light chain variable region.
28. The adzyme of claim 1, wherein said substrate is a receptor ligand, and said  
targeting moiety includes a ligand binding domain of a cognate receptor of said  
ligand.
- 25 29. The adzyme of claim 1, wherein said targeting moiety is an artificial protein or  
peptide sequence engineered to bind to said substrate.
30. The adzyme of claim 1, wherein the substrate is endogenous to a human patient.
- 30 31. The adzyme of claim 30, wherein the effect of the adzyme on the substrate is not  
significantly affected by the presence of an abundant human serum protein when  
tested with a concentration of the substrate that is about 0.5 to 2 times the expected  
physiological concentration of substrate and a concentration of the abundant  
35 human serum protein that is about 0.5 to 2 times the expected physiological  
concentration of the abundant human serum protein.

32. The adzyme of claim 31, wherein the abundant human serum protein is human serum albumin.
33. The adzyme of claim 1, wherein said adzyme alters the half-life of the substrate in vivo.
34. The adzyme of claim 1, which alters an interaction between the substrate and a receptor.
35. The adzyme of claim 1, wherein said product of said chemical reaction is an antagonist of said substrate.
36. The adzyme of claim 35, wherein said antagonist of said substrate competes with said antagonist for receptor binding.
37. A pharmaceutical preparation comprising the adzyme of claim 1 and a pharmaceutically effective carrier.
38. The pharmaceutical preparation of claim 37, formulated such that autocatalytic proteolysis of the adzyme is inhibited.
39. The pharmaceutical preparation of claim 38, further comprising a reversible inhibitor of said protease domain.
40. The pharmaceutical preparation of claim 39, wherein the reversible inhibitor is safe for administration to a human patient.
41. An adzyme for inhibiting receptor-mediated signaling activity of an extracellular substrate polypeptide, the adzyme being an immunoglobulin fusion complex comprising: a first fusion protein bound to a second fusion protein, wherein the first fusion protein comprises a constant portion of an immunoglobulin heavy chain and a protease domain that catalyzes the proteolytic cleavage of at least one peptide bond of the substrate polypeptide so as to inhibit the receptor-mediated signaling activity of the polypeptide, and wherein the second fusion protein comprises a constant portion of an immunoglobulin heavy chain and a targeting domain that reversibly binds with an address site on said substrate polypeptide, wherein said targeting domain and said protease domain are discrete and heterologous with respect to each other.